Position paper on a strategy to distribute banana (*Musa*) germplasm with endogenous Banana streak virus genomes

Prepared by a task force assembled through the MusaNet Conservation Thematic Group, after a workshop held in Montpellier, France on 5 May 2015. *Members of the task force: J Thomas[1], M-L Iskra-Caruana[2], P Lava Kumar[3], N Roux[4], M Chabannes[2], S Massart[5], Y Mathieu[6], R Chase[4] and I Van den houwe[7]*

Purpose: The purpose of this position paper is to propose a strategy for the distribution of germplasm containing endogenous BSV alleles present in the B genome, while minimising any risks associated with the distribution of BSV to the recipient country.

Keywords: endogenous Banana streak virus, episomal infection, indexing, plantain, banana, germplasm exchange

1. Background

Most banana (*Musa* spp.) cultivars have diploid, triploid or tetraploid genomes derived from intra- and inter-specific crosses of *Musa acuminata* (A) and/or *Musa balbisiana* (B). While wild *Musa* spp., are generally seeded, cultivated bananas are vegetatively propagated and movement of this germplasm is a potential avenue for the concomitant transfer of contaminating viruses. *In vitro* cultures are the preferred means of distribution of germplasm, and all viruses recorded from banana are potentially transmissible in this manner. International germplasm collections, such as the Bioversity International *Musa* Germplasm Transit Centre (ITC) at KU Leuven (http://bit.ly/1P33Bpb), Belgium and the banana germplasm collection at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria index their material to verify that it is free from viruses before distribution.

If infected accessions are identified, *in vitro* meristem micropropagation combined with therapy procedures (chemo-, thermo- or cryo-) is usually effective for the removal of contaminating viruses. Banana streak viruses (BSVs) are a group of related viruses which cause banana streak disease and are frequent contaminants of *Musa* germplasm. BSVs pose a unique problem. Not only can they be present as episomal[8] infections but viral genomes of some BSV species are also naturally integrated into the *Musa* B genome, and are known as endogenous BSV (eBSV) sequences. Under certain stress conditions (e.g. during cross hybridization, tissue culture, or abiotic stress), some eBSVs can be activated and give rise to functional viral genomes that trigger BSV infections (episomal infection). Thus a symptomatic infection can occur suddenly in a plant previously identified as BSV-free, and the virus particles are transmissible by mealybug vectors in the same way as other episomal BSV infections. With accessions carrying activateable eBSV, it is therefore not possible to guarantee BSV-free planting material.

As a consequence, many *Musa* accessions with the B genome and carrying activated eBSVs are not distributed under the current policy adopted by the ITC. For instance, about 300 of nearly 1500 *Musa* accessions in ITC are known to be affected by activated eBSVs. This includes germplasm for breeding and evaluation, artificial hybrids with valuable disease resistance and agronomic traits, and

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6 Vitropic, France
7 Bioversity International, KULeuven, Belgium
8 Episomal replication: Extrachromasomal, autonomous replication of DNA i.e. DNA replicating independently of the chromosome.

some standard reference cultivars used in the MusaNet Taxonomic Reference Collection (http://bit.ly/1E5ULR2).

However, evidence from research over the past decade has demonstrated that *Musa* spp. with eBSV are widely distributed and the corresponding BSVs are presumed to be present in almost all *Musa* production regions. This led to debate on the risks vs benefits of sharing *Musa* germplasm with activateable eBSV. Arguments favouring distribution suggest that eBSV germplasm poses no new risk to importing countries and that the benefits outweigh perceived risks. An opposing view highlights the conscious distribution of potentially BSV-infected germplasm. A summary of recent research on BSV and eBSV, and references relevant to this topic, appear below (Section 3.2, Appendix 2).

The first version of this Position Paper was posted on MusaNet (www.musanet.org) for comments and feedback from 21 August to 30 September 2015. Relevant feedback was taken into consideration in preparation of the second (current) version.

2. Previous discussions and workshops to address the eBSV issue

Over the last decade, discussions have been held on the risks of distributing germplasm containing eBSVs and arguments in favour or against the exchange of *Musa* accessions with B-genome have been put forward. An international discussion to address the impact of eBSVs on the distribution of germplasm was commenced at the 2007 ISHS-ProMusa Symposium in White River, South Africa (Appendix 1). Since then, considerable advances have been made in our understanding of eBSV (see Section 3.2 and Appendix 2), warranting the revisiting of the moratorium on the distribution of BSV-infected germplasm from the ITC. To this end, a workshop entitled "Banana streak viruses and their impact on the use of germplasm" was held at the International Horticultural Congress/ISHS-ProMusa Symposium in Brisbane, in August 2014 (summary presented in Appendix 3). This workshop was organised through the MusaNet Conservation Thematic Group, and had the following aims:

1. To identify and discuss barriers caused by eBSV on the distribution and use of genetic resources by the community, and to propose possible solutions to the issues, and
2. To make recommendations to collection holders, and especially the ITC, on a strategy to facilitate distribution of germplasm harbouring eBSV.

The workshop recommended gathering a small panel of specialists to advise Bioversity International, managing *Musa* collections at ITC, and other genebanks holding *Musa* germplasm such as IITA, on ways to best characterize accessions (BSV status and propensity for eBSV activation) and to develop a strategy for responsible distribution of all germplasm.

To address this recommendation, a panel of specialists (task force) was gathered and a workshop was then organized in Montpellier on 5th of May 2015.

3. Workshop at Bioversity International, Montpellier, 5 May 2015

The specific aims of the Montpellier workshop were:

a. Determine the extent of the problem caused by restrictions on the movement of germplasm containing eBSV and BSV.

b. Clearly elaborate the need for a change to current restrictions on distributing the *Musa* germplasm with eBSV.

c. Recommend a strategy for allowing movement of accessions containing eBSV with minimal, acceptable risk including full disclosure of its health status to recipients.

3.1 Extent of the problem and need for a change of policy

- At present, about 300 of nearly 1500 accessions at ITC are unavailable for global distribution due to episomal infections derived from eBSV detected during the virus indexing. This includes germplasm for breeding and evaluation, artificial hybrids with valuable disease resistance and agronomic traits, and some standard reference cultivars used in the MusaNet Taxonomic Reference Collection (http://bit.ly/1E5ULR2)

- Elimination of episomal BSV, whatever the origin (eBSV or non-eBSV), can be achieved, most readily through the use of meristem culture and virus therapy. However, this procedure is only effective in the long term for non-eBSV derived episomal infections because episomal infections due to eBSV activation can occur recurrently.
- Most eBSV activation occurs in the early proliferation stages of tissue culture.
- Rates of activation of eBSV vary widely across accessions.
- Reports of activation of eBSV in field situations are very limited. Examples of field spread of BSV via mealybug vector transmission are rare and not well documented (see citations in Appendix 2).
- Almost all records of BSV in germplasm are of Banana streak OL virus, Banana streak IM virus, Banana streak GF virus and Banana streak MY virus, which are present as eBSV in almost all cultivars containing the B genome.
- When present, banana streak disease symptoms are most commonly chlorotic streaks on the leaf lamina, but less commonly various forms of plant and fruit distortion and internal pseudostem necrosis leading to plant death may occur. However, symptom expression is often sporadic and dependent of the virus species, growing conditions and the plant genotype. Symptoms can be very variable in type and severity, and infected plants are frequently symptomless.

Summary of relevant research findings on BSV

Salient information from research on BSVs in *Musa* is summarized below. Most was obtained since the 2007 ISHS-ProMusa Symposium and provides the background on which the strategy for distributing germplasm containing infective eBSV in the B genome is based.

- Known to occur in almost all the *Musa* production zones around the world.
- High genetic diversity observed in BSVs.
- Phylogenetically, BSVs are distributed over Clades 1 and 3 of the badnavirus genus.
- BSVs from Clade 1 are distributed worldwide and some (BSOLV, BSIMV, BSMYV and BSGFV) have eBSV counterparts within the B genome.
- Clade 2 encompasses integrated (non-infectious) badnaviral sequences detected in both A and B genomes of *Musa* spp., with no BSV counterpart reported.
- BSVs from Clade 3 represent species from East Africa with no viral integrant (no eBSV) in the *Musa* genome reported.
- BSOLV, BSGFV and BSIMV species each show a single allelic integration (eBSV) into the diploid *Musa balbisiana* genome of PKW (BB) with one allele responsible for the infection (termed the infectious allele).
- Specific PCR and dCAPs markers have been developed for each eBSV allele (infectious and non-infectious for both BSOLV and BSGFV) which can be used for eBSV genotyping.
- Improved B genitors devoid of infectious eBSV alleles identified by marker aided selection (MAS) have been obtained by CIRAD and are now being used as genitors for breeding.
- Diagnostic procedures to distinguish eBSV from episomal infection have been well established for virus indexing and selection of episomal BSV-free germplasm.
- By the end of 2016, CIRAD will have screened all B-genome containing accessions in the ITC collection for the presence of infectious and non-infectious eBSV alleles.
- Procedures to eliminate episomal BSV infections in *Musa* have been standardized.

3.2 Workshop conclusions and recommendations

eBSVs are a natural component of almost all accessions with the B genome, and thus these accessions will always have at least the propensity for eBSV activation when they harbour infectious eBSV alleles, leading to episomal BSV infection. Although some B genome-containing germplasm without infectious eBSV alleles is now available, these accessions are very limited in number. Many useful accessions, including pre-existing artificial hybrids, contain infectious eBSV alleles, but the advantages of utilising this material must be balanced against the risks associated with its responsible distribution.

The taskforce developed a strategy to facilitate the indexing and distribution of *Musa* germplasm in relation to BSV (see below). The strategy involves the elimination of episomal infections, most importantly of non-integrated BSV species, before germplasm transfer. Non-integrated BSV species are limited in their geographical distribution and can be effectively removed.

4. A strategy for BSV indexing to facilitate the distribution of *Musa* germplasm

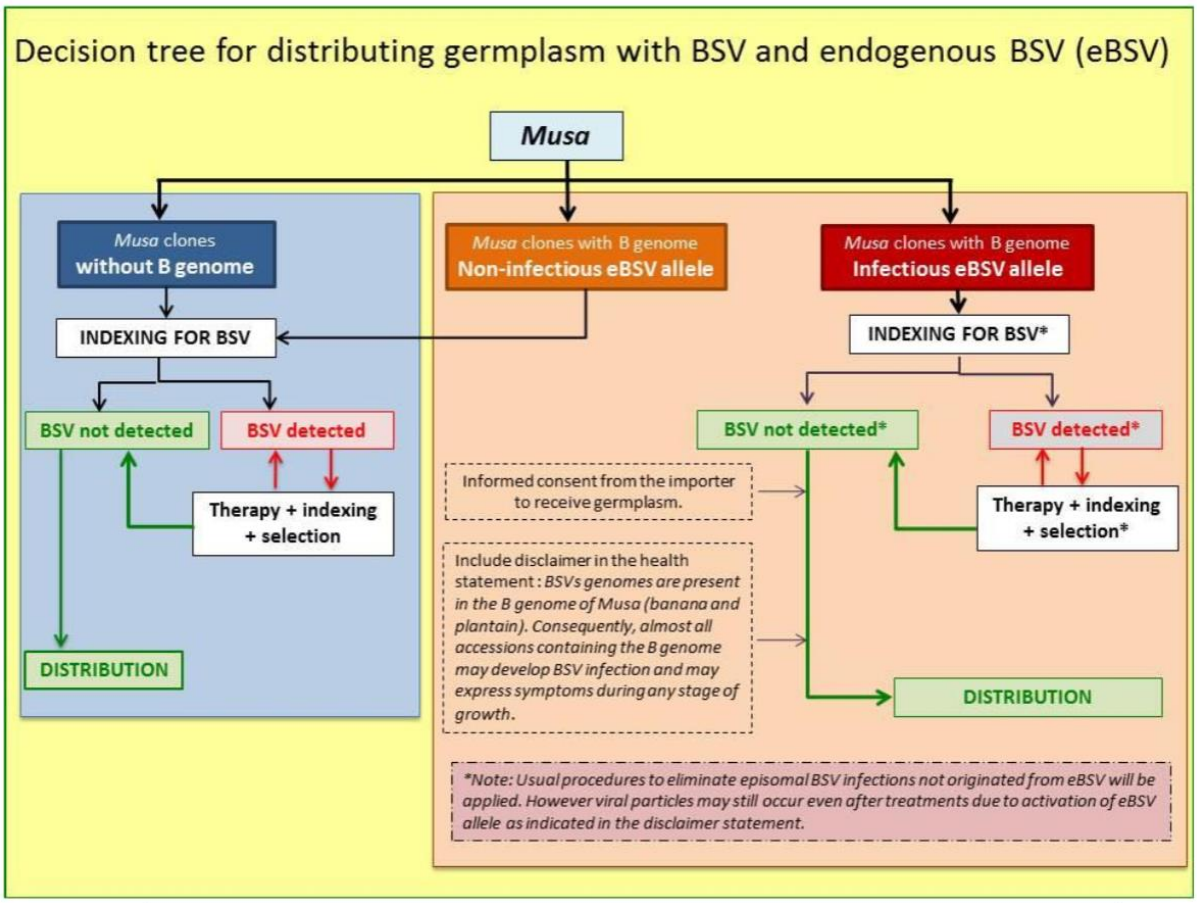
The taskforce has developed a decision tree to facilitate the indexing and distribution of *Musa* germplasm (see Figure 1). The taskforce differentiates between endogenous and non-endogenous BSV. Non-endogenous BSVs should be removed from germplasm through virus therapy before distribution. The use of virus-therapy procedures is mandatory to eliminate all BSV infections, whatever their origin, and particularly to ensure elimination of the non-eBSV. The indexing protocols used must also be able to differentiate active infections (episomal form) from endogenous viral DNA including eBSV. Although the physical indexing and therapy treatments of accessions are the same for all three categories, the decision tree highlights specific points relevant to accessions containing eBSVs. The ability to distinguish between those containing non-infectious or infectious eBSV allows greater confidence to be assigned to the risk of distribution associated with various accessions.

Three categories of accessions are considered when indexing for BSV:

1. ***Musa* accessions without the B genome:** If indexed free of BSV, they can be distributed. If BSV is detected, they can undergo virus therapy and if free of BSV upon reindexing, they can be distributed.
2. ***Musa* accessions with the B genome and only non-infectious eBSV:** If indexed free of BSV, they can be distributed. If BSV is detected, this will be very likely due to episomal infections not derived from eBSV, and they can undergo virus therapy. If free of BSV upon re-indexation, they can be distributed.
3. ***Musa* accessions with the B genome and infectious eBSV**: If indexed free of BSV, they can be distributed. If BSV is detected, this can be of either non-eBSV origin or eBSV derived BSV or both. These accessions will undergo virus therapy to eliminate all BSV particles to be sure that episomal infection of non-eBSV origin is eliminated prior to distribution.

When indexed free of BSV and other known viruses, accessions in categories 1 and 2 are available for distribution. For accessions in category 3, it is proposed that the National Plant Protection Organisation (NPPO) of the importing country should be notified of the proposed importation of any eBSV-containing germplasm, and their written acceptance of this importation must be obtained before distribution. The health statement attached to the germplasm will include the following disclaimer: e*BSVs are present in the B genome of* Musa *(banana). Consequently, almost all accessions containing the B genome may develop BSV infection and may express symptoms during any stage of growth.*

The taskforce noted that all other standard procedures in use for virus indexing and therapy to ascertain planting material freedom from known-viruses are to be followed as usual; including the Technical Guidelines for the Safe Movement of *Musa* Germplasm (MusaNet, 2015


Decision tree for distributing germplasm with BSV and endogenous BSV (eBSV)
Musa
Musa clones without B genome
INDEXING FOR BSV
BSV not detected
BSV detected
Therapy + indexing + selection
DISTRIBUTION
Musa clones with B genome Non-infectious eBSV allele
Musa clones with B genome Infectious eBSV allele
INDEXING FOR BSV*
BSV not detected*
BSV detected*
Therapy + indexing + selection*
Informed consent from the importer to receive germplasm.
Include disclaimer in the health statement : BSVs genomes are present in the B genome of Musa (banana and plantain). Consequently, almost all accessions containing the B genome may develop BSV infection and may express symptoms during any stage of growth.
DISTRIBUTION
*Note: Usual procedures to eliminate episomal BSV infections not originated from eBSV will be applied. However viral particles may still occur even after treatments due to activation of eBSV allele as indicated in the disclaimer statement.

Appendix 1

Banana streak viruses and the distribution of genebank material: prologue to a ProMusa workshop

A blog post published by Anne Vezina on the ProMusa blog on 14 Aug 2014

http://www.promusa.org/blogpost364-Banana-streak-viruses-and-the-distribution-of-genebank-material-prologue-to-a-ProMusa-workshop

One of the workshops to be held during the IHC2014 ProMusa symposium will discuss the problem banana streak viruses (BSV) pose to the distribution of genebank material. It will pick up on a discussion started by Australian virologist Andrew Geering at the 2007 ISHS-ProMusa symposium. Geering argued that the distribution of germplasm should be guided by the International Plant Protection Convention (IPPC), which states that it is the responsibility of the importing country, not of the exporter (in this case the ITC[1]), to impose phytosanitary measures. In 2008, he reprised his arguments on the old ProMusa website. He was joined by Pierre-Yves Teycheney, a virologist at the French Agricultural Research Centre for International Development CIRAD who argued against relaxing the ITC guidelines. Their arguments are reproduced below, but first some background information.

(Andrew Geering) BSV form a complex of species that cause streak disease in bananas - the main species being Banana streak Obino l'Ewai virus (BSOLV) and Banana streak Goldfinger virus (BSGFV). The viruses are transmitted by mealybugs and infected planting material, but unlike other banana viruses, and the vast majority of plant viruses for that matter, they are also integrated in the genome of *Musa balbisiana*, the wild banana species that donated the so-called B genome to many cultivars. Under certain circumstances, such as tissue culture and hybridization, integrated sequences can excise themselves and produce infectious particles. Therapies exist to clean plantlets of the autonomous viral particles, called episomal, but not of the integrated sequences. So when an ITC accession that has the B genome tests positive for the episomal form of BSV, it is not cleaned to make it available for distribution, as would happen for accessions that don't have the B genome, since the plantlets may produce BSV viral particles when they are tissue cultured. Under the current guidelines, infected accessions that belong to the AAB, ABB or AAAB genome groups, with Plantains (AAB) being the most affected group, are not available for distribution. In 2008, these represented about 20% of the accessions at the ITC.

At the time, Geering argued that the ITC should make an exception for material infected with BSV, as long as approval is granted by the relevant plant protection authority in the importing country, whereas Teycheney argued that there is not enough information to make an informed decision. Here are their arguments.

The argument for relaxing the ITC guidelines by ***Andrew Geering***

Principles guiding the international movement of plants and plant products

The IPPC is the principal treaty by which countries abide to help prevent the spread of pests (including pathogens) in plants and plant products. As of 2 August 2007, there were 163 contracting governments to the IPPC. The IPPC is governed by the Commission on Phytosanitary Measures, which prepares International Standards for Phytosanitary Measures (ISPM) in order to achieve international harmonization of quarantine policies and to facilitate trade by preventing countries from using unjustifiable measures as trade barriers.

Under the IPPC, an importing country has the right to impose phytosanitary measures for regulated pests only, whether they are quarantine or non-quarantine pests. A quarantine pest is defined as "a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled". A regulated non-quarantine pest is defined as "a non-quarantine pest whose presence in plants for planting affects the intended use of those plants with an economically unacceptable impact and which is therefore regulated within the territory of the importing contracting party". The IPPC further stipulates that the phytosanitary measures must also not be any more stringent than those presently in place if the pest is already in the country. It is the duty of the importing country to publish and transmit their phytosanitary requirements, which may require consignments to enter through specified points of entry if the imports need to be inspected, treated or accompanied by a phytosanitary certificate. The importing country can also "make special provision, subject to adequate safeguards, for the importation, for the purpose of scientific research, education, or other specific use, of plants and plant products and other regulated articles, and of plant pests".

Pest risk analyses

One of the fundamental principles of the IPPC is managed risk, recognising that there is always a risk of spread and introduction of pests when importing plants and plant products and that importing countries should only institute phytosanitary measures consistent with the pest risk involved. The technical tool used to identify appropriate phytosanitary measures is a pest risk analysis (PRA). A PRA may be initiated when "there is an intention to import for selection and/or scientific research a plant species or cultivar not yet introduced that could potentially be a host of pests" (ISPM No. 2 – 2007). It is the responsibility of the importer to prepare the PRA and to communicate any recommendations from this PRA to the exporter.

In preparing a PRA, a number of factors should be considered including the category of the pest, the economic impact of the pest, the potential for establishment and spread of the pest, and the proposed uses of the plants or plant products. "The conclusion of the pest risk management stage will be whether or not appropriate phytosanitary measures adequate to reduce the pest risk to an acceptable level are available, cost-effective and feasible" (ISPM No. 2 – 2007). If the pest risk is considered unacceptable and there are no measures available to mitigate the risk, then the import can be prohibited. At the other end of the spectrum, if the pest risk is considered negligible, the import may be permitted with few if any phytosanitary measures.

Pest categorisation

The first stage of a pest risk analysis is to identify pests that may require phytosanitary measures. As previously mentioned, an importing country can only impose phytosanitary measures for regulated pests (quarantine or regulated non-quarantine pest) but not for non-regulated pests (i.e. pests that are indigenous or introduced and widespread). It is beyond the scope of this paper to provide advice on which category BSOLV and BSGFV may fall into in the different banana-producing countries but some general comments can be made.

BSOLV and BSGFV, being endogenous badnaviruses linked to the B genome of *Musa*, can be expected to be found in any country where *Musa* A×B hybrids are grown, and therefore are unlikely to be categorised as quarantine pests anywhere in the world. In some countries, these viruses may already be abundant and widespread in distribution and therefore classified as non-regulated pests. Both BSOLV and BSGFV have been recorded in Australia, but are restricted in distribution and are under active control, and therefore would be categorized as regulated non-quarantine pests.

Recommendations There are probably many instances where the benefits of distributing BSOLV- or BSGFV-infected accessions from the ITC to partner organizations or growers may far outweigh the risks. Examples include:

(i) Export of accessions to regions where the viruses are already well-established and widespread.

(ii) Export of accessions for experimental purposes where the viruses could be contained by geographic isolation or by growing them in a glasshouse.

(iii) Export of accessions to countries where economic gains from use of the accessions may far outweigh losses caused by the virus (e.g. if the accessions have resistance to black leaf streak disease or Fusarium wilt).

(iv) Export of accessions to countries where the risk of spread and establishment of the viruses is low (e.g. where the rate of mealybug spread is very slow).

It is the responsibility of each respective national plant protection organization to determine whether an importation of BSOLV- and BSGFV-infected plants into a country should be allowed and what phytosanitary measures should be imposed. If permission has been granted, there is no basis under the IPPC for Bioversity International to restrict movement of the plants from the ITC. The only obligation on Bioversity International is to ensure the accuracy of the information and additional declarations contained in phytosanitary certificates and to follow any requested phytosanitary measures (ISPM No. 1 - 2006).

The argument against relaxing the guidelines by ***Pierre-Yves Teycheney***

Several endogenous banana streak virus sequences (eBSVs) are present in the nuclear genome of *Musa balbisiana*, hereafter referred to as the B genome. So far, no germplasm containing the B genome has been found to be free of eBSVs. The discovery that some eBSVs can be activated to cause infection in AAB and AAAB genotypes following in vitro culture or genetic crosses has lead CIRAD to introduce a moratorium (i) on the use of *M. balbisiana* for breeding new interspecific hybrids and (ii) on the distribution of plant material harbouring the B genome. This moratorium is based on the precaution principle to avoid spreading infectious BSV species resulting from the activation of eBSVs.

Only two potentially infectious eBSVs have been identified so far in the B-genome. Nevertheless, considering the diversity of BSV species and that of the eBSVs characterized so far, it is expected that more eBSVs, including activateable ones, are present in the B genome.

The main risk associated with eBSVs is the introduction in farmers' fields of infectious viral particles following the wide distribution of B-containing germplasm whose integrated sequences have been activated by in vitro culture or abiotic stresses, such as changes in temperature or water stress. Such outbreaks could be amplified by the natural transmission of viral particles by mealybugs, whose population dynamics remain largely unknown. Therefore, several countries do not allow the importation of plants and plant products infected with BSV species. Under French quarantine regulations, for example, the only exemptions are in vitro banana plantlets derived from *Musa acuminata*, which may be brought into French overseas departments subject to quarantine and virus indexing for a number of viruses, including banana streak viruses.

Why not recommend the export of B-containing germplasm to regions where BSV species are already well established and widespread?

It is likely that the B genome contains more activateable eBSVs than those characterized so far. Exporting B-containing germplasm, even to regions where known BSV species are present, might promote the diffusion of new BSV species and should be prohibited until the sequencing of the *M. balbisiana* nuclear genome has been completed, leading to the characterization of all eBSVs.

Why not recommend the export of B-containing germplasm for experimental purposes where BSV species could be contained by geographic isolation or by growing them in a glasshouse?

Importation of quarantine pathogens for experimental purposes is possible under tightly regulated conditions established by plant protection and quarantine services of importing countries. B-containing germplasm should be considered as potentially infected and handled accordingly, unless BSV species as a whole are not considered a quarantine pathogen by relevant authorities. Containment by geographical isolation is not an option unless the surveillance of experimental plots can be guaranteed to avoid the dissemination of the plant material.

Why not recommend the export of B-containing germplasm to countries where economic gains would outweigh losses caused by BSV species?

Several disease-resistant AAB and AAAB interspecific hybrids have been successfully bred by FHIA's breeding programme in Honduras. They have been widely distributed in Africa, Central and South America and the Caribbean even after activation of eBSVs under stress conditions had been experimentally demonstrated for at least one of these hybrids, FHIA-21. It is now established that all AAB or AAAB interspecific hybrids can become infected following stress-induced activation of eBSVs. Although BSV infections have been reported in areas where in vitro plantlets of AAB or AAAB hybrids have been distributed, no statistically supported data are available on the impact the distribution of these hybrids has had on the spread of BSV. It is therefore currently impossible to evaluate this risk and decide on a scientific basis whether the benefit of using AAB or AAAB hybrids outweighs the risk of spreading BSV. This type risk/benefit analysis needs to be undertaken to inform decisions on the matter.

Why not recommend the export of B-containing germplasm to countries where the risk of spread and establishment of BSV species is low?

Data on the epidemiology of BSV is very scarce and inexistent with regards to the population dynamics of its insect vectors. Therefore, it is currently impossible to decide whether and where "the risk of spread and establishment of BSV species is low" and to design forecasting models to assess this risk. This shortage of information currently prevents the identification of low-risk countries and areas.

Conclusion

There is a growing need to provide developing countries with higher-yielding as well as disease- and stress-resistant Musa genotypes. Unfortunately, the presence of eBSVs in the B genome is a major constraint to the mass multiplication and distribution of germplasm containing the B genome. The CIRAD moratorium on this type of germplasm was decided in order to prevent the spread of BSV. It was initially planned to revisit it when sufficient scientific data on the activation of eBSVs have been gathered and fighting strategies have been developed and implemented. Despite major scientific breakthroughs in the past five years, the genetic and molecular mechanisms underpinning the activation of eBSVs still need to be unravelled. Moreover, little efforts have been made to assess the risks of spreading BSV through the distribution of B-containing germplasm. There are currently not enough data to justify relaxing the ITC guidelines regarding the distribution of BSV-infected

germplasm. In order to resolve this critical issue without taking inconsiderate risks, there is an urgent need to assess the risk of spreading BSV species through such a distribution. This will lead to guidelines for the safe movement and distribution of B-containing germplasm, and might possibly also lead to the restoration of the use of *M. balbisiana* in breeding programmes.

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1. Each accession that is introduced to the ITC is inspected for virus symptoms and undergoes extensive testing for Banana bunchy top virus (BBTV), Cucumber mosaic virus (CMV), Banana bract mosaic virus (BBrMV), Banana mild mosaic virus (BanMMV) and banana streak viruses (BSV) and previously unrecognised viruses... It is not available for distribution until it has received a clean bill of health

Appendix 2: List of relevant literature on eBSV/ BSVs

Chabannes M., Baurens F.-C., Duroy P.-O., Sidibe-Bocs S., Vernerey M.-S., Rodier-Goud M., Barbe V., Gayral P., Iskra-Caruana M.-L. (2013). Three infectious viral species lying in wait in the banana genome. Journal of Virology 87 (15): 8624-8637.

Chabannes M., Iskra-Caruana M.-L. (2013) Endogenous pararetroviruses – a reservoir of virus infection in plants. Virus replication in animals and plants. Current Opinion in Virology 3 (6): 615-620.

Côte F., Galzi S., Folliot M., Lamagnère Y., Teycheney P.Y., Iskra-Caruana M.-L. (2010). Micropropagation by tissue culture triggers differential expression of infectious endogenous Banana u of endogenous sequences of Banana streak virus: what can we learn from banana (Musa sp.) evolution? Molecular Plant Pathology 11: 137-144.

Gayral P., Blondin L., Guidolin O., Carreel F., Hippolyte I., Perrier X., Iskra Caruana M.-L. (2010). Evolution of endogenous sequences of Banana Streak Virus: what can we learn from banana (Musa sp.) evolution? Journal of Virology 84 (14): 7346-7359.

Gayral P., Iskra-Caruana M.-L. (2009). Phylogeny of Banana streak virus reveals recent and repetitive endogenization in the genome of its banana host (Musa sp.). Journal of Molecular Evolution 69 (1): 65-80 h

Geering ADW, Thomas JE (2002) Banana streak virus. AAB Description of plant viruses 390.

Iskra-Caruana M.-L., Chabannes M., Duroy P.-O., Muller E. (2014). A possible scenario for the evolution of Banana streak virus in banana. Virus Research 186:155-162.

Iskra-Caruana M.-L., Duroy P.-O., Chabannes M., Muller E. (2014) Different partners involved in a common story. Infection, Genetics and Evolution 21:83-89.

Iskra-Caruana M.-L., Baurens F.C., Gayral P., Chabannes M. (2010). A four-partner plant-virus interaction: enemies can also come from within. Molecular Plant-Microbe Interactions 23 (11): 1394-1402.

Kumar, P.L., Selvarajan, R., Iskra-Caruana, M-L., Chabannes, M. and Hanna, R. (2015). Biology, etiology and control of virus diseases of banana and plantain. Advances in Virus Research 91: 229-269.

Lassois, L., Lepoivre, P., Swennen, R., Van den houwe, I., Panis, B. (2013). Thermotherapy, chemotherapy, and meristem culture in banana. In: Lambardi, M., Ozudogru, A.E., Jain, S.M. (eds) Protocols for Micropropagation of Selected Economically-Important Horticultural Plants. Springer Science+Business Media New York, pp 419-433.

Lockhart BEL, Jones DR (2000) Banana streak. In: Jones DR (ed) Diseases of banana, abacá and enset. CAB International, Wallingford, pp 263-274

Meyer, J.B., Kasdorf, G.G.F., Nel, L.H., Pietersen, G. (2008) Transmission of activated-episomal Banana streak OL (badna)virus (BSOLV) to cv. Williams banana (*Musa* sp.) by three mealybug species. Plant Disease 92:1158-1163.

More literature on banana streak viruses in Musalit:

www.musalit.org/saveSearch.php?id=f39b865df68363cfe4fc665a4e580ac9

Appendix 3

BSV Workshop Report, Brisbane August 2014

International Horticultural Congress, Brisbane 2014

"BANANA STREAK VIRUSES AND THEIR IMPACT ON THE USE OF GERMPLASM"

Symposium Workshop Tuesday 19th August 2014

MusaNet Conservation Thematic Group, Bioversity International

Banana streak viruses (BSV) constitute a major impediment to the distribution and use of *Musa* germplasm around the world, due to:

- The presence of endogenous, activateable viruses in the B genome
- Activation of infections from the B genome, especially after environmental stresses and tissue culture.
- Widespread occurrence in *Musa* germplasm
- Existence as a "cryptic" species with considerable genomic diversity
- Complexity of detection assays and necessity of distinguishing endogenous from exogenous sequences.

This workshop was organized by John Thomas (University of Queensland, Australia), chair of the MusaNet Conservation Thematic Group. It was the continuation of a discussion started by Australian virologist Andrew Geering (University of Queensland, Australia), and followed up by CIRAD virologist Pierre-Yves Teycheney, at the 2007 ISHS-ProMusa symposium, which was summarized in a ProMusa blog. Developments in our understanding of the molecular structure of integrated BSV prompted a further examination of this subject.

Workshop aim:

The objectives of the workshop were to:

1. identify and discuss barriers caused by BSV on the distribution and use of genetic resources by the community, and to propose possible solutions to these issues.
2. make recommendations to collection holders, and especially ITC, on strategies to facilitate distribution of BSV-infected germplasm.

Workshop format:

The workshop was chaired by John Thomas (University of Queensland, Australia). Short presentations were made as situation statements on the following subjects:

- current needs for germplasm that is restricted by BSV integration/activation issues (Edson Amorim / EMBRAPA, Brazil)

- risks associated with distribution of this germplasm (Andrew Geering)
- responsibilities of importers and exporters of the germplasm (Bart Panis, Bioversity International)
- possible alternatives to using this germplasm (Pierre-Yves Teycheney)

These short presentations were followed by a roundtable discussion on these issues and possible ways to overcome restrictions on the use of *Musa* germplasm. Over 30 participants from a variety of disciplines (including virologists, breeders, germplasm curators, horticulturists and program managers) attended the workshop.

Presentations

The presentations are available on the ProMusa website (http://www.promusa.org/article117-IHC-2014-Australia#workshops).

John Thomas outlined the background to the workshop.

Andrew Geering gave an overview of the properties of BSV, its taxonomy and mealybug transmission. He noted the paucity of data on the impact of BSV on production, with most available information being anecdotal. BSV is a major contaminant of international germplasm, with the majority of accessions with the B genome being infected and the activateable viruses already widely distributed internationally. He concluded by noting that under international conventions, it is ultimately the importing country that bears the responsibility for the material it imports.

Pierre-Yves Teycheney described the directions CIRAD was taking within their breeding program to address the BSV issue. The two avenues are being explored at CIRAD are:

- Risk assessment of spreading BSV through large scale distribution of existing interspecific hybrids, and
- Creation of improved *M. balbisiana* genitors devoid of infectious eBSV

The latter is possible since the elucidation of the structure of BSV integrants and the ability to differentiate infectious and non-infectious eBSV. This work by CIRAD is the major advance since the ProMusa workshop in 2007.

Edson Amorim noted that the AAB cultivars predominate in Brazilian production. The EMBRAPA germplasm collection is being evaluated for its integrated BSV status, with the cooperation of CIRAD. Symptom expression in the field is not common, but sometimes occurs after environmental stress.

Bart Panis outlined the structure and function of the *in vitro* germplasm collection held at the Bioversity International Transit Centre (ITC). He presented information on virus indexing and virus therapy, and noted the policy of the ITC only to provide material that has indexed virus-free.

Discussion

General importance of BSV in the field

The discussions were wide ranging. It is evident that there is limited documented and published data on the impact of BSV on production (e.g. Daniells et al 2001; Lassoudière 1974, 1979; Murekezi 2005)[1] In addition there are several examples of unpublished observations and anecdotal data. Jerome Kubiriba (NARO) and Anthony James (QUT) mentioned a limited outbreak of BSV in germplasm imported from ITC and IITA. This comprised about 20 symptomatic plants out of a few hundred, and consisted of eBSV species only, sometimes as mixed infections. BSMYV was found in germplasm but not in the field. Thierry Lescot (CIRAD) gave several examples of BSV outbreaks in Costa Rica, Colombia, Peru and Ecuador. In most cases these were B genome-containing cultivars,

but one example, from Ecuador, was Cavendish infected with BSOLV. He and Brian Irish (USDA, Puerto Rico) both mentioned the variability in levels of activation of endogenous BSV under different levels of stress (e.g. FHIA 21). Uma Subburaya (NRCB, India) also noted a progressive yield decline and environmental effects on symptoms in cv. Mysore, infected with BSMYV.

A wide range of symptomatology was also described, with fruit symptoms sometimes occurring, including irregular bunch emergence, thin, splitting peel, mosaic patterns and necrotic spotting. However, the appearance of these symptoms in both leaves and fruit is erratic. It is most likely that only the worst cases of BSV infection are noticed and publicised, and less severe cases go unrecognised or unreported.

Miguel Dita (EMBRAPA, Brazil) raised the question about the effect of individual BSVs on a range of individual cultivars, and it became apparent that very little is known in this area. A major practical consideration is working with cultivars containing the B genome, where inoculation with a specific BSV could be confounded by uncontrolled activation of eBSVs.

[1] Daniells et al (2001) Annals of Applied Biology 139: 51-60; Lassoudière (1974) Fruits 29: 349-357; Lassoudière (1979) Fruits 34, 3-34; Murekezi, C. (2005) Ph.D.thesis, Reading (U.K.).

Current status of BSV-infected accessions in collections

At the ITC, accessions with active, episomal infections, either from field infection or activation of an endogenous allele, are held in quarantine. Cultivars lacking the B genome (no activateable endogenous BSV) are subjected to virus elimination therapy, which if successful allows release of the germplasm.

At CIRAD, natural hybrids (local cultivars) that have indexed virus-free are screened with molecular markers for endogenous BSV, and then micro-propagated and reintroduced to the country of origin of the planting material. The importer is informed about the possible BSV expression due to infectious eBSV when it exists in the B genome of the cultivar For newly created hybrids having B genomes, CIRAD breeders are now working again with interspecific hybrids and seeded BB diploids having either non-infectious eBSV or no eBSV, and thus posing no risk of BSV activation.

At IITA, the Inter-African Phytosanitary guidelines for *Musa* germplasm exchange in Africa are followed:

"(i) On the exchange of *Musa* spp Internationally, and recognising that the BSVDNA are integrated to the *Musa* chromosomes, and BSV being worldwide in distribution, newly bred *Musa* hybrids can be exchanged without restrictions among interested countries.

(ii) The exporting agency must however, ensure that all procedures for virus testing of *Musa* germplasm are complied with and only virus tested free materials are exchanged."

Consequences of maintaining the status quo

- Unavailability of some existing hybrids and landraces for field assessment (e.g. Taxonomic Reference Collection, provitamin A assessment), use as breeding parents and distribution to endusers (e.g. cultivars with black Sigatoka and panama wilt resistance). As an example, of 300 plantain accessions held in the ITC, only 20 (<10%) are virus-free.
- Cost of maintaining *in vitro* germplasm. The question arises that if the material is to be stored indefinitely with no hope of distribution or virus therapy, should it be discarded? But if rare genotypes held *in vitro* are not maintained, this will ultimately lead to an erosion of genetic resources and reduction of diversity in the field

Consequences of distributing eBSV-containing accessions

- Possible outbreaks of BSV in new plantings. The risk of activation is dependent on both cultivar and environmental and physiological stresses. Some hybrids such as FHIA 21 seem particularly prone to BSV activation. Some hybrids e.g. Goldfinger (FHIA 001, AAAB) and natural selections e.g. Ladyfinger (AAB) in Australia virtually never express BSV.
- New BSV species could potentially be imported with germplasm. However the eBSVs, especially BSOLV, are the most common BSVs internationally already.
- In most circumstances, natural spread of BSV via insect vectors is extremely slow, making control through eradication of infected plants a practical consideration. If an accession demonstrates lower levels of activation, then infected plants could be removed and replaced, with negligible risk to other nearby plantings

Means of limiting or avoiding the negative effects of BSV

- Fred Bakry (CIRAD) described a strategy of sourcing germplasm and shipping back to the same country with natural hybrids and landraces. Experience in West Africa indicated only low levels (e.g. 4%) of tissue culture derived plants expressing BSV symptoms. This method presents no additional threat to the importing country, but utilizes only the germplasm already in that country.
- Virus elimination through meristem tip culture and chemotherapy, cryotherapy or electrotherapy as undertaken for the ITC collection. This is only feasible in non-B genotypes, but would limit the distribution of the less common BSVs that are not integrated,
- Conventional (non-tissue culture) multiplication of planting material, such as macropropagation, to avoid activation during tissue culture

- Use of B genome genitors lacking activateable eBSV. This is a major advance for future breeding programs. However, a problem is the small percentage of B genome-containing hybrids lacking activateable eBSV and the small number of activateable eBSV-free hybrids that can be obtained through artificial crosses. This means that a limited range of B genome diversity will be available for breeding.

Proposed ways forward

- Wait until new CIRAD hybrids are released. This is a very safe approach, but will result in long delays before new hybrids are available and a more limited B genome diversity in the hybrids.
- Release "quarantined "germplasm with activateable BSV. This will result in the availability of a much wider range of genotypes and diversity. Is it possible to release this material with a statement of its eBSV molecular status and possibly its propensity for activation under a standard set of environmental conditions (e.g. after micropropagation, compared to a reference cultivar)?
- Release limited quantities of germplasm for conventional *in situ* propagation and eventual wider release of individual plants not expressing BSV. Macropropagation methods may be appropriate here.

- If they are not to be distributed in the short to medium term, BSV-infected accessions could be held in cryopreservation only, thus significantly reducing the cost of storage.

Observations and Recommendations

- Further controlled and well-documented field studies are required on the economic impact of BSV.
- Responsibility for the importation of *Musa* germplasm rests with the importing country.
- The exporter is obliged to fully describe the material being exported.

  The International Standards for Phytosanitary Measures, ISPM 7, Phytosanitary Certification System (2011) states:

  "The NPPO of the exporting country has the sole authority to undertake phytosanitary certification..."

  "Phytosanitary certification should be based on official information from the importing country. The NPPO of the exporting country should, to the extent possible, have available current official information concerning the phytosanitary import requirements of relevant importing countries."

  In the case of BSV, could this Phytosanitary Certification include a "health statement" which could mention the status of eBSV and likelihood of activation?
- The workshop did highlight a need for the distribution of some BSV-infected and quarantined accessions from ITC.
- It would be useful for a small panel of specialists to be brought together to advise Bioversity International on ways to best characterize accessions and report their BSV status and propensity for activation, and thus allow a mechanism for responsible distribution of all germplasm.

- If a modest number of cultivars were targeted for conditional release, it may be possible to map their integrants, and also grow out a standard number of tissue cultured plantlets under standard conditions to assess the relative rate of activation compared e.g. to a reference hybrid.